acetylcholine receptor-channels (AChRs) indicates that a conformational change in the protein is an essential part of the ligand association process, and that this structural rearrangement shares a common mechanism with the ensuing low-to-high affinity transformation of the binding site that triggers channel-gating. The energy source for gating is the change in affinity for the agonist. We find that in AChRs there is a close correlation between the low- and high-affinity equilibrium association constants. This indicates that agonist association and the low-to-high affinity-change that powers gating are linked processes that reflect a single, integrated 'catch-and-hold' structural adaptation of the protein to the ligand. The slope of the correlation, kappa, is an index of the relative position in this reaction co-ordinate, which occurs in the initial stages of the gating isomerization. Residue GlyB2 changes energy ('moves') early in the process (k=0.85), before the agonist and four a-subunit aromatic amino acids ($k \approx 0.5$). In 1957 del Castillo and Katz separated binding and gating. The results suggest that they are just different stages of a single reaction co-ordinate.

**604-Pos Board B390**

**Variations in Binding Amongst Several Agonists at Two Stoichiometries of the Neuronal, α4β2 Nicotinic Receptor**

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Nicotinic acetylcholine receptors (nAChR) are pentameric ligand-gated ion channels found throughout the central and peripheral nervous systems. Neuronal α4β2 receptors play a prominent role in nicotine addiction, and they exist in two stoichiometries: $(\alpha4)_2(\beta2)_3$ termed high affinity and $(\alpha4)_3(\beta2)_2$ termed low affinity. Herein, we evaluate several key drug-receptor binding interactions of four agonists - ACh, nicotine, and the smoking cessation compounds varenicline (Chantix®) and cytisine (Tabex®) - at both stoichiometries of the α4β2 nAChR. Previous studies have established that unnatural amino acid mutagenesis can probe the three key binding interactions at the nAChR: a cation-π interaction, and two hydrogen bonding interactions to the protein backbone of the receptor. We find that all drugs make a cation-π interaction to TrpB (αW154) of the receptor. All drugs except ACh, which lacks an $N^+H$ group, make a hydrogen bond to a backbone carbonyl, and ACh and nicotine behave similarly in acting as a hydrogen bond acceptor. However, varenicline is not a hydrogen bond acceptor to the backbone NH that interacts strongly with the other three compounds considered. In addition, we see interesting variations in hydrogen bonding interactions with cytisine that provide a rationalization for the stoichiometry selectivity seen with this compound.

**605-Pos Board B391**

**Genistein's Effects on Alpha7 nAChR Currents Depends upon Exposure Duration and Time Course of Expression in *Xenopus* Oocytes**

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Several previous studies that explored tyrosine phosphorylation/dephosphorylation-involvement in functional upregulation of α7-nAChRs found that genistein, a broad-spectrum inhibitor of protein tyrosine kinases (PTKs), increased Ach-evoked α7-nAChR currents. Interpretation of genistein's effects as due to perturbation of tyrosine-phosphorylation processes is complicated, however, by subsequent findings that genistein is a strong positive allosteric modulator (PAM) of α7-nAChRs. Our study asked if genistein's enhancement of α7-nAChRs currents is entirely attributable to PAM activity, or if non-PAM effects contribute as well. We compared the effects of brief (1 min) vs. extended (24 h) exposure of oocytes expressing α7-nAChRs to genistein (100 μM), both 2 and 5 days following cRNA injections (2.0 ng). On day 2, the Ach-evoked (500 μM) peak currents for control cells, and cells exposed for 1 min, or 24 h, to genistein were (mean ± SEM, nA): 29.32 ± 12.10 (n=24), 130.95 ± 35.42 (n=22), and 374 ± 105.21 (n=8). On day 5, the comparable values were: 108.61 ± 14.85 (n=10), 310.81 ± 56.22 (n=9), and 605.00 ± 128.03 nA (n=11). On day 2, 1 min genistein caused a 4.5 fold-facilitation; 24 h exposure caused a 12.8 fold-facilitation. On day 5, 1 min genistein caused a 2.4 fold-facilitation; 24 h genistein caused a 5.6 fold-facilitation. Thus, genistein-enhancement of α7 currents varied inversely with the degree of functional α7-nAChR expression, being greater for day 2 than 5. Genistein's effect on desensitization of α7-nAChR currents was also separable from facilitation. On day 2, genistein increased peak current without affecting desensitization. On day 5, genistein increased peak current and slowed desensitization. Our results suggest that genistein's enhancement of α7-nAChR currents is not entirely due to PAM effects, but involves other mechanisms too, particularly on day 2.

## Mechanosensitive Channels

**606-Pos Board B392**

**Clustering of the Mechanosensitive Ion Channels of Large and Small Conductance MscL and MscS - a FRET-Flim Study**

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MscL channels preferentially cluster in bacterial cells *in vivo* [1]. Recently it was shown that MscL channels also cluster when reconstituted into liposomes suggesting that this phenomena is bilayer mediated [2]. Evidence was based on atomic force microscopy, small angle neutron scattering, confocal microscopy and patch clamp electrophysiology. However doubts remained as to whether or not proteins were introduced into the liposomes as a cluster, or whether they in fact self-assemble into clusters. In this study, we used FRET FLIM microscopy to address this problem in more detail and compared clustering of MscL with itself, MscS with itself, and MscL with MscS. Separate populations of MscL and MscS channels were labeled; one population with an Alexa Fluor 488 FRET donor and the other with an Alexa Fluor 568 FRET acceptor. Samples were reconstituted into the lipids separately and the 2 samples were allowed to mix overnight. FLIM images of donor fluorescence in the mixed lipid-protein samples were collected. Reduced donor fluorescence lifetimes, due to FRET, indicated clustering between the 2 protein populations. Fast protein liquid chromatography suggested that a portion of MscL and MscS proteins will cluster and/or form higher order oligomers in detergent buffers. These fractions were excluded from the FRET-FLIM experiments. We demonstrated that MscL will self-assemble into clusters in liposomes, and also demonstrated that MscS and MscL will co-cluster. However, there was no evidence for MscS clustering with itself. Clustering of channels is of significance as channel activity is thought to be modulated by neighboring proteins [2].

*We acknowledge the NH&MRC (Grant 635525). E.D. acknowledges the UWA convocation.*

[1] Norman, C., *et al.* (2005)*Eur Biophys J* 34: 396-402.
[2] Grage, S.L., *et al.* (2011)*Biophys J.* 100: 1252-1260.

**607-Pos Board B393**

**The Dynamics of Protein-Protein Interactions Between Cytoplasmic Domains in MscL**

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The bacterial Mechanosensitive channel of large conductance, MscL, is one of the best characterized mechanosensitive channels serving as a paradigm for mechanosensitivity. MscL's physiological role is that of an emergency release valve that opens upon a sudden drop in the osmolarity of the environment. A crystal structure of a closed state of MscL revealed that the protein exists as a homopentamer, with each subunit consisting of two transmembrane domains with both N and C-terminal domains cytoplasmic. Since the size of the open pore is approximately 30Å, massive structural changes have to occur upon gating. There is consensus that the transmembrane helices move in an iris like manner tilting in the plane of the membrane while gating, but little is known about protein-protein interactions between the two cytoplasmic regions adjacent to the transmembrane domains. The N-terminal amphipathic helix, which lies along the membrane, and the cytoplasmic portion of TM2 and adjacent regions are crucial for channel gating, and some residues are predicted to be in close proximity in the crystal structure. Here we aim to determine how these two regions interact in the channel complex, and to study how these interactions may change as the channel opens. By using an *in vivo* disulfide trapping approach, we have searched for specific protein-protein interactions that occur in different states (closed, open and transition) of *E. coli* MscL channel gating. We have screened 143 double-cysteine mutants for their efficiency in disulfide bridging and generated a map of protein-protein interactions between these two cytoplasmic regions. A set of interesting candidates have been studied by patch clamp and show differences in channel activity under different redox potentials.